A Pre-synopsis entitled as

“Bio-Management of Major Diseases of Soybean by using Bacteria from Rhizospheric Niches”

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Introduction:

Soybean (*Glycine max L. Merrill*) is an important crop in the worldwide and also in India, as it plays a crucial role in the human consumption up to 25% of the global nutritive oil. Soybean comprises 40 % proteins, 20% oil and also a good source of essential amino acids. (Jaybhay, *et al.*, 2018). soybean oil also used in the production of biodiesel, biopolymer, industrial chemical, human and animal feed. As the soybean is the leguminous plant it improves fertility of the soil, by fixing nitrogen through symbiotic nitrogen fixation with nitrogen fixing bacteria lives in root by forming root nodules. Use of soybean majorly done for the oil and protein production.

The major soybean producing nations are the United States, Brazil, and Argentina. These three countries dominate global production, accounting for 80% of the world's soybean supply. Soybean contributes significantly to the Indian edible oil pool. Presently soybean contributes 43 % to the total oilseeds and 25% to the total oil production in the country. Currently, India ranks fourth in respect to production of soybean in the world. The crop helps earn valuable foreign exchange by way of soya meal exports. Soybean has largely been responsible in uplifting farmer's economic status in many pockets of the country. It usually fetches higher income to the farmers owing to the huge export market for soybean de-oiled cake (https://ficci.in/spdocument/20539/soybean-report.pdf).

India has the fifth largest vegetable oil economy in the world. After cereals, oilseeds are the second largest agricultural commodity, accounting for the 14% of the gross cropped area in the country. However, country meets its edible oil demand through imports, which accounts for almost 50% of the requirement. The per capita consumption of the vegetable oil is increasing very rapidly due to increase in population and improved economic status of the population. The demand has increased to about 12.6 kg/year compared to 4 kg/year in 1961 and the projected demand for the year 2020 and 2050 is 16.443 and 19.16 kg/year respectively. To meet this demand, the country will require nearly 25.26 and 35.90 million tons of edible oil. In this scenario, soybean has played and will play a pivotal role in the future. Production of soybean in India is dominated by Maharashtra and Madhya Pradesh which contribute 89 per cent of the total production. Rajasthan, Andhra Pradesh, Karnataka, Chhattisgarh, and Gujarat contribute the remaining 11 per cent production. Maharashtra has the potential of becoming the leader in Soybean. Having realized the fact that new technologies for sustainably increasing the crop yields are essential, PPPIAD project takes a holistic approach to provide end-to-end solutions to the Soybean growing farmers in Maharashtra. (https://ficci.in/spdocument/20539/soybean-report.pdf).

Review of Literature:

Although there are majorly increase in the global production of the soybean there are different challenges for the production of soybean may include biotic and abiotic constraints. Abiotic factors like low availability of fertile soil, poor nodulation, seed longevity are vital difficulties in tropics, and biotic factors like diseases, insect pests and weeds cause a major challenge in the yield of the soybean (Murithi *et al.,* 2016).

Diseases of Soybean:

Different organisms cause infections in the soybean like bacteria (bacterial Blight caused by *Pseudomonas savastami pv glycinea*, bacterial pustule caused by *Xanthomonas axonopodis pv glycines*), fungi (Anthracnose caused by *Colletotrichum truncatum* and several other species, Brown spot caused by *Septoria glycines, Cercospora* leaf blight and Purple seed stain caused by *Cercospora kikuchii,* Downy mildew caused by *Pernospora manshurica,* Frogeye Leaf Spot caused by *Cercospora sojina*. Charcoal Rot caused by *Macrophomina phaseolina* , Stem Canker caused by *Diaporthe phaseolorum var. meridionalis*, Phytophthora Root Rot causal agent *Phytophthora sojaeanf Fusasrium spp.*, Sudden Death Syndrome caused by *Fusarium virguliforme*, Alternaria Leaf Blight caused by *Alternaria spp*.) and Viral like Soybean Mosaic Virus, Bean Pod Mottle Virus (Faske *et al.,* 2014).

Alternaria Leaf Blight of Soybean:

Alternaria species mostly are saprophytic and derive their energy from decaying dead plant tissues by cellulolytic ability. Although most saprophytic species are infectious cause disease when they acquire capacity to cause a disease. They have wide range of hosts and cause disease in variety of important plants including cereals, ornamentals, oil crops, vegetables like cabbage, cauliflower, broccoli, carrot, and potato. They also cause diseases in fruits like tomato, citrus apple (Leuven, 2003). *Alternaria* produces asexual spore called conidiophores which gives rise to conidia. *Alternaria* species are foliar pathogens, they destruct the tissue of host and reduces the photosynthetic ability of the plant which leads to the formation of necrotic lesions (Leuven, 2003). In soybean it causes Alternaria leaf spot disease having symptoms are like round lesions, or restricted by major vein or merged in another lesion (Huilgol *et al.,* 2020). As this is the secondary invader causing disease only after the mechanical injury in the soybean but it has ability to cause loss upto 10 to 25 % under harsh conditions (Huilgol *et al.,* 2020), due to ability of forming asexual conidiophores *Alternaria* grows at high humid, low temperature 16 $^{\circ}$C to 24 $^{\circ}$C, hence the disease is severe when favorable conditions are formed (Mamgain *et al*., 2013).

Fusarium Wilt of Soybean:

Fusarium spp. is a filamentous fungi cause root rot a disease commonly found in many crops, having symptoms like round or irregular light brown lesions that grows in to dark black lesions on below ground roots and stems (Bodah, 2017). *Fusarium oxysporum* and *Fusarium solani* are generally responsible for the cause of Root rot in soybean, but also other species may also cause the disease like *F. armeniacum, F. graminearum, F. proliferatum, F. pseudo- graminearum,* and *F. redolens* (Arias *et al.,* 2013). Different diseases are commonly caused by the *Fusarium* species like sudden death syndrome caused by *F. virguliforme* also common in many crops and soybean also (Okello *et al.,* 2020). The disease root rot was seen in many countries and causing upto 47 to 55 % loss in many countries (Arias *et al.,* 2013; Okello *et al.,* 2020).

Many studies found that the incidence was increased under stressful conditions. The fungus colonize within the roots and damage the internal vascular system of the plant (Jimenez, 2017). *Fusarium* infects the plants in both vegetative and reproductive stage of plant, and gives symptoms, and hence there is need to control the disease with major attention (Arias *et al*., 2013). Diseases caused by fungi are also a majorly related to environmental conditions and speciation. The diseases are also controlled by maintaining both physical and cultivation practices, it involves management or cultivation of disease resistance plant varieties, use of artificial fungicides, organic product produced by plant like plant extracts, biostimulator (Jain, *et al*., 2019).

Enhancing the productivity of crop, it is necessary to control the disease on primary level of disease. hence the disease is primarily controlled by using synthetic chemicals. Study shows that the use of fungicide diminish the ability of plant to photosynthesize, and plant physiology, it also enter in the water bodies remain entire season in the water and harms to the aquatic ecosystem (Dias, 2012; Zubrod *et al.,* 2019). It is also found that the some parasitic fungi which naturally inhibit the growth of pests and acts as biological controlling agents but due the nonspecific activity of chemical fungicides also kills these entomopathogenic fungi (Fiedler and Sosnowska, 2017).

The different side effects of synthetic fungicides nowadays limits their use and intensify need to search an ecofriendly method to control the severity of the disease by using biocontrol agents (Didwania and Solanki, 2013). Whipps, (2001) reports that the soil contains millions of microorganisms which lives in different types of association among them such as mutualistic, symbiotic, antagonism etc. The bacteria living immediate vicinity to the roots of plants is known as rhizobacteria and the zone known as rhizosphere. The rhizobacteria play a

many vital roles for the increasing plant growth and development and hence known as plant growth promotors. These plant growth promoting rhizobacteria (PGPR) promote the growth by producing phytohormone, biological nitrogen fixation, enhancing bioavailability of nutrients, biocontrol of phytopathogens (Ashraf *et al.,* 2013). The study of Whipps (2001) shows that PGPR induces systemic resistance in crops against a number of pathogens and also produces toxic metabolites, sequestrating iron by siderophore production, antibiotic production, antifungal enzymes e.g., chitinase production.

From all above studies, we are finally designed to target the Alternaria leaf spot and Fusarium wilt disease on the soybean by using the antifungal property of rhizobacteria from soil. Hence the present work mainly focused on the biological control of *Alternaria spp.* and *Fusarium spp.* on the soybean by isolating the rhizobacteria from soil associated with soybean.

Objectives of Research:

Soybean crop shown higher yield in the last decade but due to the soil deterioration and change in the microbial community in the crop leads to the development of many diseases which cause the losses in yield in upcoming days. The use of synthetic chemicals leads to the many health perspectives in animals and humans also, hence it is in need to find out an alternative way to reduce the use of synthetic chemicals for the crop protection and increase the productivity. This study mainly focused to find out the antifungal bacteria against the *Alternaria spp.* causing leaf blight and *Fusarium spp.* causing Fusarium wilt of the soybean. The chronology of the study is designed given below:

1. To isolate the Phytopathogen of Alternaria Blight and Fusarium Wilt from the Infected Soybean crop.
2. To isolate the Rhizobacteria from the soil samples
3. To Screen rhizospheric isolate for antifungal activity against phytopathogens.
4. To identify the efficient bacterial isolates by 16S rRNA sequencing.
5. To detect the biocontrol mechanism of Rhizospheric isolates.
6. To reveal the growth promotion abilities of efficient biocontrol isolates.
7. To prepare the formulation of efficient isolates.
8. To perform the pot assay for biocontrol and growth promotion abilities of selected isolates.

Methodology:

1. Isolation of Phytopathogen from the Infected Soybean crop:

Isolation of *Alternaria* Blight pathogen:

Infected leaves of Soybean plants showing the characteristic symptoms of Alternaria leaf blight disease collected in sterile polyethylene bags from different districts of Marathwada region and brought to research laboratory. Leaves cleaned under tap water to remove phylloplane organisms. Each diseased leaf cut into small pieces (5-10 mm), washed with sterile distilled water and surface sterilized with 0.1 % mercuric chloride for two minutes. Surface sterilized tissues rinsed with three changes of sterile distilled water to remove the traces of $HgCl_2$. Four pieces transferred on PDA medium with sterile forceps in 90 mm Petri-dishes and then incubated at room temperature for 6-7 days (Raut and Hamde, 2019).

Isolation of *Fusarium* wilt Pathogen:

Fusarium wilt infected Soybean plants collected from different fields of Marathwada region in sterile polyethylene bags and brought to research laboratory. Infected stems and branches of Soybean washed with sterile water thoroughly. The infected stem tissues cut into small pieces (2–5 mm size) and by using flame-sterilized forceps; they were transferred to sterile Petri plates containing 0.1 % mercuric chloride solution for 30-60 s. These surface sterilized pieces of stem tissues again transferred to another sterile Petri plates containing sterile distilled water to remove the disinfectant solution with 2-3 changes of sterile water. The outer layer of tissues removed rapidly with sterile surgical blade and small pieces from the central core of tissues in the advancing margin of infection cut and aseptically transferred to Petri plates containing Potato Dextrose Agar (PDA). Petri plates incubated at room temperature for 6 days (Raut and Hamde, 2016). Isolated pure culture of phytopathogen maintained on PDA and Kings- B agar plate and also on slant for further use.

2. Isolation of the Rhizobacteria from the Soil Samples:

Rhizospheric soil samples from healthy Soybean growing fields of different districts of Marathwada region collected in sterile polyethylene bags and brought to research laboratory. 1g of each rhizospheric soil sample mixed with 100 ml sterile distilled water and shaken well for 2 min, and then the content of flask allowed to settle. Different dilutions 10^{-1}, to10^{-6} of these samples prepared by serial dilution technique. Highest dilution 10^{-4}, 10^{-5} and 10^{-6} dilutions used for plating propose. 0.5 ml selected dilutions plated by using pour plate technique. All the plates incubated at room temperature for 24-48 h (Raut and Hamde, 2016).

3. Screening of Antifungal activity against Phytopathogens.

The rhizobacteria isolates screened for antifungal activity against phytopathogen by using dual culture technique on King B agar plates (Gull and Hafeez, 2012).

4. Identification of Efficient Bacterial Isolates:

An effective antifungal rhizospheric bacterial isolates obtained through screening against Phytopathogens, identified with the help of 16S rRNA sequencing.

5. Biocontrol mechanism of Rhizospheric isolate:

While finding the mechanism of the microbial control agent, the rhizospheric isolates tested for the production of volatile metabolite, diffusible metabolite and siderophore production etc.

6. Revealing of Growth Promotion Abilities of Efficient Biocontrol Isolates.

The efficient Biocontrol cultures were evaluated for various plant growth promoting characters, such as HCN production (Lorck, 2004), ammonia production from organic compound using Nessler's reagent (Cappuccino and Sherman, 2005), IAA production using 1% tryptophan (Brick *et al.,* 2004) and Phosphate solubilization on Pikovskaya's medium (Pikovaskaya, 1948).

7. Preparation of formulation for the isolates.

Carrier based powder formulations of microbial control agents will be prepared and used for further study (Rakh, 2010).

8. Pot Assay for Biocontrol and Growth Promotion:

Using pot assay, the biocontrol and growth promotion efficiency of the selected isolates carried out as per the (Rakh, 2010).

Importance of the study:

Soybean crop most commonly used for the production of oil and protein both for the consumption of human and animal feed also. Within the small timeframe it acquires the huge market and dominated all other oilseed for oil and protein production. It also used in the biodiesel, biopolymer and industrial chemicals (Gaonkar and Rosentrater, 2019). Soybean (*Glycine max*) generally grown all over the world, it acquires generally 6 % of the worlds cultivable land (Murithi *et al.,* 2016). In Maharashtra, soybean is cultivated on an area of 3.8 M ha with production of 3.07 m ton with second rank in India. However, low productivity of the crop remains a major problem of soybean cultivation, necessitating the need to ascertain the problems faced by soybean growers (Jaybhay *et al.,* 2018). The study of Jaybhay *et al.,* (2018) found that labour problems, irrigation facilities, weed management, marketing of produce, pest and disease management were the top five constraints faced by the farmers in

soybean cultivation. Among the five major constraints of soybean grower farmers, the diseases caused by Fungi, bacteria and viruses also plays a major role in limiting the yield of soybean. There are many different fungal pathogens can cause the disease like, *Alternaria* leaf blight caused by *Alternaria alternate*, charcoal rot caused by *Macrophomina phaseolina*, Downy mildew caused by *Peronospora manshurica*, *Fusarium* root rot caused by *Fusarium solani* and many more (Li & Yang, 2009).

Besides the all importance of this crop the poor productivity and heavy difference in climate decreases the yield due to the detrimental effects (Agarwal *et al*., 2013). Studies also shows that the temperature changes also favor the growth of fungus like *Alternaria alternata* grows at 35^0C and optimum pH at 7.5 to 6.5 (Gawai and Mangnalikar, 2018). To control these diseases, there are different synthetic antifungal fungicides are available in the market like penthiopyrad, propiconazole plus chlorothalonil, (Grichar and Woodward, 2016), Mancozeb, Hexaconazole and pyraclostrobin (Shamurailatpam and Kumar, 2020).

Although the use of these fungicide are effective against fungal disease but they also generates great harmful effects to humans, animals, and environment .Hence there is need to find the alternative measure to control these diseases which could be ecofriendly as well economical (Shamurailatpam and Kumar, 2020).

Many biocontrol agents known so far which gives better results in controlling the disease and nonharmful. Soil contains a numerous bacterium which live in specific association with these phytopathogens having mutualism or antagonistic effect. These microbes secrete secondary metabolites and generate a relation among them it may be positive or negative. Microbes associated with the plants roots establishes positive association and responsible for growth of both plants as well as microbes with equal benefits and hence known as Plant growth promoting rhizobacteria (Tabassum *et al.,* 2017). Soil contains many different bacteria associated with plants which secretes antifungal compounds like antibiotics, biosurfactants, hydrogen cyanide, lytic enzymes also generates a systemic response against the pathogens. (Tabassum *et al.,* 2017).

Hence the present study endeavors to find the alternatives to synthetic fungicides which would be ecofriendly, economic, and also health effective to humans as well as animals. The present study mainly focused on the biological control of phytopathogens of soybean by using the rhizobacteria.

Status of the problem:

High health hazards, detrimental effects on the soil and environment, improper use of all chemicals leads to toxicity to humans as well as animals (Shamurailatpam & Kumar,

2020), many disease causing fungi are also developing an fungicide resistance mechanisms is a major challenge for the environment (Shamurailatpam and Kumar, 2020; Roth *et al.,* 2021). Alternaria leaf spot disease becoming the major problems in soybean growing in Karnataka (Huilgol *et al.,* 2020). Many diseases are yet identified caused by the fungi which cause serious problems in the recent crops not only in the soybean but also other crops also (Borah and Deb, 2020). Diseases developing in any stage of life of the crops but majorly diseases are also time specific which cause the diseases in the specific period of life of plants and also these are area specific i.e. infect specific part of the plant (Faske *et al.,* 2014; Borah and Deb, 2020). Hence to control this fungal disease need to find effective methods but along with them it is to be remembering that these methods should not be harmful to the environment or animals and humans.

The antagonistic properties of plant growth promoting bacteria are well known against the fungi. Hence the here new approach to control the disease is evolved in which microbes are used to control the fungal phytopathogens. The mechanisms in which microbes are used to control of the disease, the concept is known as biocontrol of diseases. So, to tackle these problems in soybean, we are endeavored to find for effective biocontrol agents in the form of rhizobacteria.

International Status:

Internationally several researchers have reported use of biocontrol agents for the management of soybean diseases. Some of them mentioned as:

Soybean (*Glycine max L. Merrill*) is an economically commodity for all over the world, also in USA but the productivity in USA reduced by diseases upto 51% due to the various fungal diseases like cyst nematodes, charcoal rot, seedling diseases. Bandara *et al* (2020) concluded in their metadata analysis that cumulative loss due to the fungal diseases is higher and tend to be increased from 2005 to 2016.

Viriyasuthe *et al.,* (2019) studied the biocontrol of *Alternaria* by using the *Trichoderma* and found that there is greater result as equal with the fungicide sprays (propiconazole) but no harm to the other pathways but inoculation with the *Trichoderma* increase the propagules formation, controls the growth of *Alternaria* on the crop.

Attia *et al.,* (2020) shows that PGPR isolated from the rhizospheric soil gives effective antagonistic potential against *Alternaria solani* causing leaf blight disease in tomato plant and gives insights of solving major serious problems and challenges against the *A. solani* invasion.

National status:

At the National level, on these phytopathogens *Alternaria* leaf blight and Fusarium wilt of Soybean no work has been done on the biocontrol aspect.

Sen *et al.,* (2012) reported that Fluorescent *Pseudomonas* BRL-1 isolated from the rhizosphere, showed both in vitro and in vivo antagonistic activity against the *Alternaria alternata*. In dual culture bioassay as circular and semicircular patterns, the isolate quantitatively inhibits the growth of the pathogen by about 78% and 69%, respectively. In vitro plate assay detected that the strain produces hydrolytic enzymes, IAA and siderophore.

Kendre et al., (2020) reported that the 15 Bacillus isolates inhibited the growth of all foliar pathogens including *Myrothecium roridum*, *Colletotrichum dematium*, *Alternaria alternata* (soybean), *Colletotrichum truncatum*, *Curvularia lunata*, *Phoma medicaginis* and *Alternaria alternata* (cotton), in the range of 76.13 – 85.59% against PBs13 isolates. The minimum growth inhibition (81.81%) of *Myrothecium roridum, Colletotrichum dematium, Alternaria alternata* (soybean), *Colletotrichum truncatum, Curvularia lunata, Phoma medicaginis* and *Alternaria alternata* (cotton) was recorded in *B. subtilis* isolate PBs13 followed by PBs3 (72.81%), PBs14 (69.45%) and PBs4 (67.34%) respectively. The other isolates of *B. subtilis* i.e. PBs15, PBs10, PBs6 and PBs7 were found least effective against all the fungal pathogens.

Significance of the study:

Soybean (*Glycine Max L.*) is a crop grows worldwide, in India the major growing states are Madhya Pradesh, Maharashtra, Karnataka, Andhra Pradesh, Rajasthan and Chattisgarh as an important seed legume which contributes 25 % of total global edible oil, about the two third world protein for human animal and also for the poultry and fish farm. Although there huge importance and useful in direct to the health of animals, birds and human, the excessive use of chemicals for the controlling of diseases, fertilizers, pesticides leaves harmful effects among all environment components. Hence scientists are consistently trying to solve the problems and trying to find out the alternative methods to limit the use synthetic chemicals in the agricultural fields specifically in the crops which directly used for the consumption purpose.

Many researchers working on the biological entities to solve the problems associated with crop production like biofertilizers, biocontrolling of diseases, biopesticides, biofungicides etc. the proposed work is same endeavors for the finding out the alternative method for the controlling of diseases caused by the fungi, in the present we specifically isolating the bacterial Sp from soil, Rhizospheric soil, and checking the ability to control the disease Alternaria leaf blight caused by *Alternaria spp*.

Work Plan:

1 six-month	**Isolation of the Phytopathogen of Alternaria Blight and Fusarium Wilt from the Infected Soybean crop.** **Isolation of the Rhizobacteria from the soil samples**
2 six-month	**Screening of rhizospheric isolate for antifungal activity against phytopathogens.** **Identification of the efficient bacterial isolates by 16S rRNA sequencing.**
3 six-month	**Detection of the biocontrol mechanism of Rhizospheric isolates.**
4 six-month	**Revealing of the growth promotion abilities of efficient biocontrol isolates.**
5 six-month	**Preparation of the formulation of efficient isolates.** **Pot assay for biocontrol and growth promotion abilities of selected isolates.**
6 six-month	**Thesis writing and submission**

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